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Case Report

Diagnostic Challenges Related to Myeloid/Natural Killer Cells, a Variant of Myeloblasts

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Abstract: The authors report herein two diagnostically challenging cases centered on the myeloid/natural killer (myeloid/NK) cells, a variant of myeloblasts, to illustrate the importance of advanced flow cytometric immunophenotyping and an updated understanding of surface markers in hematopoietic malignancies. Myeloid/NK cell acute leukemia is a very rare subtype of leukemia. Although its NK-cell nature is debatable, it represents a variant of leukemia with distinct morphological and immunophenotypical features. The first case is a de novo myeloid/NK-cell acute leukemia with a striking clinical, morphologic and immunophenotypic resemblance to acute promyelocytic leukemia (APL), but which could be distinguished by its CD11a, CD18, CD117 and CD9 expression. This case illustrates the importance of utilizing the APL surrogate surface phenotype of HLA-DR^{low}, CD11a^{low} and CD18^{low} by flow cytometric study to rule in/out APL immunophenotypically. In the second case, we show that myeloid/NK-cell blasts can present as a variant of blasts in a preleukemic disease as refractory anemia with excess blasts-1 (RAEB-1), where the blasts were negative for CD34, CD117 and HLA-DR. The recognition of such blast variant is important in appropriately classifying such preleukemic diseases by blast percentage.

Key Words: Acute leukemia, flow cytometric immunophenotyping, myeloblast, natural killer cell

Introduction

Myeloid/natural killer (myeloid/NK) cell acute leukemia was first reported by Scott *et al* [1] in 1994 as a subtype of acute leukemia with distinct morphological and immunophenotypical features. Although its NK-cell nature is largely in debate given our recent understanding of myeloid and NK-cell differentiation, it is nevertheless characterized by a unique immunophenotype: CD33⁺, CD56⁺, CD11a⁺, CD13^{low+}, CD15^{low+}, CD34[±], HLA-DR⁻, CD16⁻, and unique morphologic features: deeply invaginated nuclear membranes, scant cytoplasm with fine azurophilic granularity, and finely granular Sudan black B and myeloperoxidase (MPO) cytochemical reactivity. These morphological features and immunophenotype are remarkably similar to those of APL, particularly the microgranular variant

(M3v). However, all the cases that were tested lacked the t(15;17) or the promyelocytic retinoic acid receptor (RAR α) fusion transcript detected by reverse transcriptase-polymerase chain reaction (RT-PCR), and failed to show a differentiation-induction response to all-*trans* retinoic acid (ATRA) *in vitro*. The treatment outcome was comparable to that expected for AML patients treated with daunorubicin and cytosine arabinoside, with a median survival of 30 months.

Following the initial description by Scott *et al*, only a few single case reports have been published [2-5]. The case reported by Lee JJ *et al* [3] represented a case of myeloid/NK leukemia developing in a patient with preexisting T-cell lymphoma. Kaya *et al* [2] described a case of myeloid/NK cell acute leukemia with an immunophenotype of CD33⁺, CD56⁺, CD16⁻, HLA-DR⁻ which was preceded by multiple myeloma. In a letter to the editor, Paietta *et al* [6] reported 2 cases of AML showing an immunophenotype of HLA-DR⁻,

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CD34⁻, CD33⁺, CD13⁺, CD56⁺, CD11a⁺ and CD16⁻, but both cases harbored the long form of the *PML/RARa* fusion transcript, suggesting that some of the myeloid/NK leukemia might be authentic M3. In addition, such variant blasts have never been described in association with other preleukemic diseases, such as a myelodysplastic syndrome (MDS).

Here we report a case of de novo acute leukemia with an immunophenotype and morphology similar to the myeloid/NK-cell acute leukemia initially described by Scott et al, and a case of refractory anemia with excess blasts (RAEB)-1 where the blasts showed myeloid/NK-cell characteristics. Both cases posed significant diagnostic challenges.

Case Report

Case 1

A 40 year old female presented with fever, chills, night sweats over a two-week period, precipitated by pleuritic chest pain and hemoptysis. She was admitted to the emergency room where she was found to have leukocytosis and thrombocytopenia. A complete blood count (CBC) showed white blood cell (WBC) $89.9 \times 10^9/L$, hemoglobin (Hb) 9.6 g/dL, platelets $73 \times 10^9/L$ with 3% neutrophils, 5% promyelocytes, 2% monocytes, and 85% "blasts". Coagulation studies revealed very high fibrin degradation products (FDP) and D-dimer, and low antithrombin III activity, indicative of pathologic fibrinolysis and severe disseminated intravascular coagulation (DIC). Peripheral smear revealed numerous immature cells with invaginated nuclear membranes, inconspicuous to prominent nucleoli, and scant to moderate hypogranular/ agranular cytoplasm (**Figure 1**).

Flow cytometric study (Beckman Coulter FC-500) performed on peripheral blood sample showed that the immature cells were positive for CD33, CD56, CD71^{low}, but negative for CD34, HLA-DR, CD117, CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD13, CD15, CD16, CD11b, CD64, CD14 and CD1a. Intracytoplasmic and nuclear stains performed by flow cytometry following permeabilization showed the blasts were positive for MPO, negative for Tdt, cytoplasmic CD3, or cytoplasmic CD22. The immunophenotype and morphology in the clinical setting of coagulopathy raised the possibility of APL, microgranular variant.

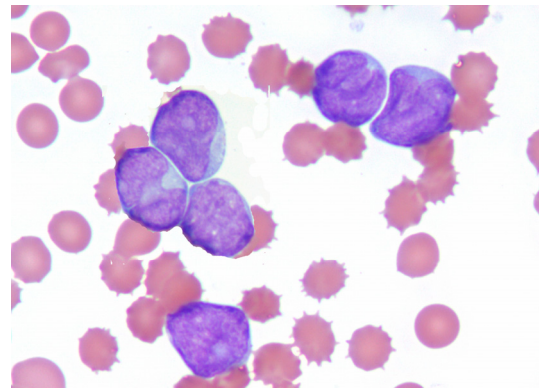


Figure 1 Peripheral blood smear showing blasts with deeply invaginated nuclear membrane and some bilobed nuclei (Wright & Giemsa stain, original magnification x1000).

Additional markers designed for APL were thus tested to show that the leukemic cells were CD11a⁺ (>85% cells), CD18⁺ (>85% cells), and CD9⁺ (50% cells). Conventional karyotyping showed a normal female karyotype. Neither t(15;17) nor other rearrangement involving *RARa* or *PML* gene was detected following fluorescence in situ hybridization (FISH) technique using DNA (ONCOR) hybridizing to *RARa* (17q12) and *PML* (15q22).

A diagnosis of myeloid/NK-cell acute leukemia was rendered. It was classified as AML-M1 according to the French-American-British (FAB) classification, and AML, not otherwise categorized (NOS)-M1 according to the world health organization (WHO) criteria. She received hydroxyurea resulting in 50% leukoreduction, followed by standard induction chemotherapy composed of 3 days of daunorubicin and 7 days of cytarabine. She achieved complete remission at day 30. Her consolidation chemotherapy consisted of mitoxantrone and VP-16, and she has been disease free 9 months after diagnosis.

Case 2

The patient was a 77 year old male who presented with fatigue to his primary care physician. His CBC showed WBC $2.0 \times 10^9/L$, Hb 9.3 g/dL; platelets $43 \times 10^9/L$ with 77% neutrophils, 1% monocytes, and 22% lymphocytes. He was referred to our medical center where a bone marrow biopsy was performed. His bone marrow showed a 70% cellularity with many dysplastic megakaryocytes (**Figure 2B**). The bone marrow

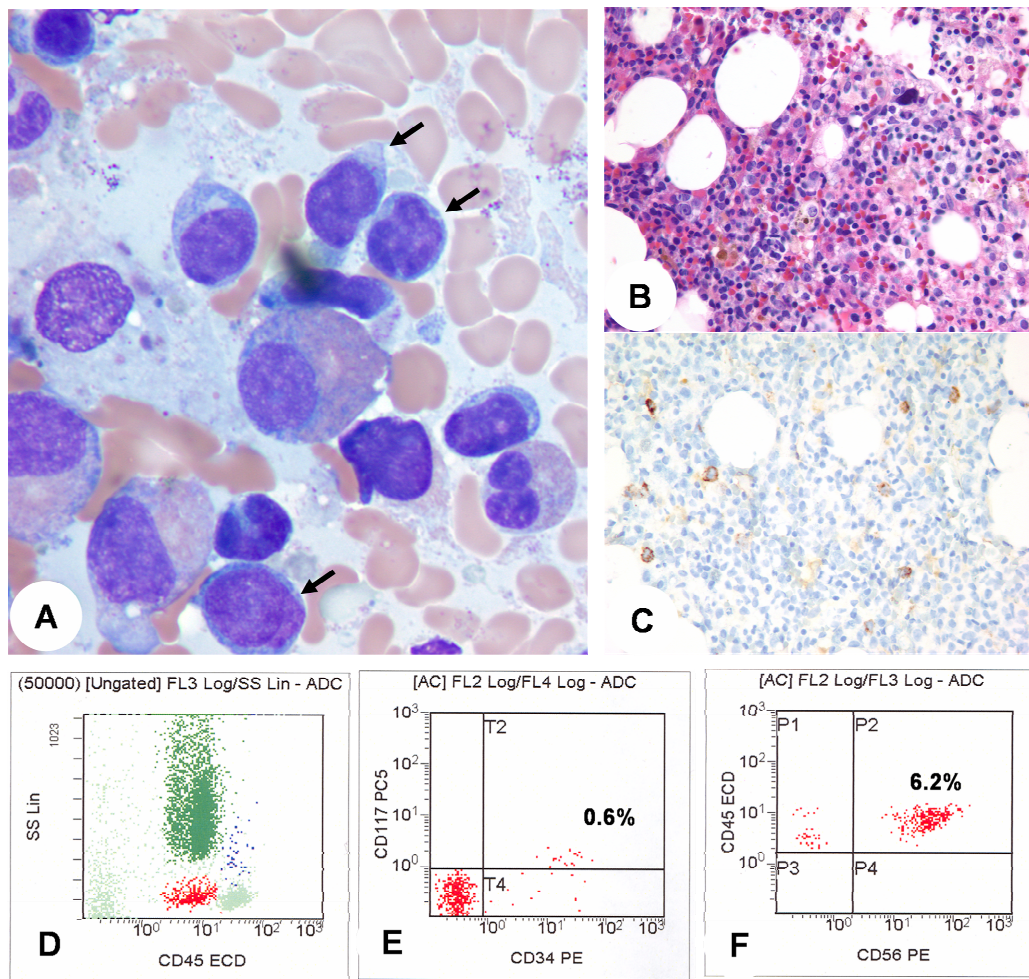


Figure 2 A. The bone marrow aspirate showed increased blasts ranged in size with invaginated/convoluted nuclear membrane, as pointed by the arrows (Wright stain, original magnification x1000). B. The bone marrow biopsy showed approximately 70% cellularity with dysplastic megakaryocytes (H&E, original magnification x400). C. CD34 immunohistochemical stain highlighted only “conventional” CD34⁺ positive blasts (original magnification x400). D-F. Flow cytometric study showed increased CD45 immature cells (highlighted in red) on side scattered vs CD45 plot (D); the “conventional” blasts (0.6%) are CD34⁺CD117⁺CD56⁻ and myeloid/NK cell blasts (6.2%) are CD34⁺CD117⁺CD56⁺ (E and F).

aspirate smears revealed increased blasts that ranged in size with hypogranular/aganular cytoplasm, and some had invaginated/convoluted nuclear membrane (**Figure 2A**). Granulocytic and megakaryocytic dysplasia was apparent. A total of 4.2% CD45^{low+} immature cells were detected by flow cytometric analysis, of which, 3% cells with an immunophenotype of CD33⁺, CD56⁺, CD34⁻, HLA-DR⁻, CD13⁻, CD117⁻, CD16⁻, CD11b⁻, CD64⁻, CD2⁻, CD3⁻, CD7⁻ and 1.2% cells with an immunophenotype of CD34⁺, HLA-DR⁺, CD117⁺, CD33⁺, CD13⁺, CD56⁻ were seen. A CD34 immunohistochemical stain was performed on bone marrow biopsy, revealing

approximately 1-2% positive cells (**Figure 2C**). Conventional karyotyping showed trisomy 8 in 2 out of 30 metaphase cells examined [47,XY,+8[2]/46,XY[28]]. Interphase FISH was performed with a probe to the chromosome 8 centromere to reveal trisomy 8 in 9 out of 100 cells. The case was classified as RAEB-1 based upon persistent clinical cytopenia, the presence of dysplasia and increased blasts. He was enrolled in a pilot study and treated with the experimental drug thalidomide and azacitidine (Vidaza). A follow-up bone marrow biopsy performed 11 months after the initial diagnosis showed a cellularity of 70-80% with similar degree of morphological dysplasia. The

bone marrow contained 8% blasts based on bone marrow aspirate differential count. Flow cytometric analysis showed 6.2% blasts with an immunophenotype of CD33⁺, CD56⁺, CD34⁻, HLA-DR⁻, CD13⁻, CD117⁻, CD16⁻, CD11b⁻ and 0.6% blasts of CD34⁺, HLA-DR⁺, CD117⁺, CD33⁺, CD13⁺ and CD56⁻ (Figures 2D, 2E and 2F). Chromosomal study performed on the follow-up bone marrow showed a normal karyotype 46, XY[20]. FISH study with a probe to the chromosome 8 centromere revealed trisomy 8 in 3/100 cells. The patients remained red cell transfusion-dependant. Seventeen months after diagnosis, he died of anemia accompanied by upper gastrointestinal bleeding. There was no evidence of acute leukemia transformation.

Discussion

Here we reported a case of acute leukemia and a case of RAEB-1, where the blasts showed typical myeloid/NK-cell characteristics as initially described by Scott et al [1]. Both cases are extremely rare and posed great diagnostic challenges.

Following the initial description by Scott et al, Suzuki et al [7] reported 7 cases of "myeloid/NK-cell precursor" acute leukemia that showed an immunophenotype of CD7⁺, CD33⁺, CD34⁺, CD56⁺, frequently HLA-DR⁺, MPO⁻ and a lymphoblastic (L2) morphology. Subsequently reported cases in the literature on myeloid/NK acute leukemia entity were mainly of the latter form, which have been found to not be an uncommon type of AML and which have been classified under M0 by FAB and WHO. Myeloid/NK precursors were also reported in Philadelphia chromosome-positive chronic myelogenous leukemia (CML) blast crisis [8, 9]. In contrast, myeloid/NK-cell acute leukemia was only rarely reported [2-5] after the initial description by Scott et al. Moreover, it has never been reported as a variant blasts in pre-leukemic diseases, such as MDS.

Out of the 158 new acute myeloid leukemia cases diagnosed at our institute over the last 5 years, our case 1 was the only case showing an immunophenotype and morphology similar to myeloid/NK-cell acute leukemia described by Scott [1]. The case showed a substantial clinical, morphologic and immunophenotypic similarity to APL. However, the current understanding of APL surface markers by flow

cytometry enabled us to differentiate APL from other CD34⁺HLA-DR⁻ acute leukemia. Paietta et al has discovered that PML/RARα^{pos} APL cells typically lack leukocyte integrins. The HLA-DR^{low}, CD11a^{low}, CD18^{low} phenotype provides a reliable surrogate antigen expression profile for PML/RARα^{pos} APL, irrespective of morphology and transcript isoform with 100% accuracy [10]. Other markers also show differential expression in APL and myeloid/NK-cell leukemia, such as CD117, which is often expressed in a high percentage of APL cases [10]. APL also often shows CD9 expression in more than 75% blasts; and CD2 co-expression has been associated with M3v morphology and/or S-isoform [10]. In our case, the myeloid NK-cells were CD117⁻, CD11a⁺ (>85% cells), CD18⁺ (>85% cells), and CD9⁺ in only 50% cells, differing from APL. Based upon this immunophenotype, the case was diagnosed as non-APL but myeloid/NK-cell acute leukemia, which should be considered as a variant AML and classified as AML-NOS-M1 according to WHO criteria. FISH confirmed the absence of any RARα gene rearrangement which also excluded APL-like subset where RARα is fused with different partners, such as promyelocytic leukemia zinc finger, nucleophosmin, or nuclear mitotic apparatus. In 2004, Kussick et al [11] described a subtype of acute leukemia that showed some morphological and immunophenotypical resemblance to myeloid/NK cell leukemia. There was a high frequency of internal tandem duplication (ITD) of the *Flt3* gene in their series. Unfortunately, there was no material remained to test for *Flt3* ITD in our case.

Case 2 illustrates that myeloid/NK-cells can be present as a variant of blasts in preleukemic disorders such as MDS. The blasts had similar morphologic features and immunophenotypic characteristics as seen in myeloid/NK-cell acute leukemia. Often, the blasts in MDS exhibit an immunophenotype of committed myeloid precursors (CD34⁺, CD38⁺, HLA-DR⁺, CD13⁺ and CD33⁺), regardless of the MDS subtype [12]. The presence of myeloid/NK blasts could cause diagnostic confusion, and result in an underestimation of the blast number, especially when the bone marrow aspirate smears are suboptimal for an accurate manual blast counting. A CD34 and/or CD117 immunohistochemical stain performed on a bone marrow biopsy specimen may be misleading. It is also important not to misinterpret basophils as myeloid/NK-blasts

because they share some immunophenotypic features with myeloid/NK blasts and can be significantly increased in preleukemic disorders, such as a myeloproliferative disease and MDS. Basophils have an immunophenotype of CD45^{low+}, CD34⁻, HLA-DR⁻, CD117⁻, CD13⁺, CD33⁺, CD18⁺, but CD11b⁺, CD56⁻, CD25⁺ and CD9⁺ [13]. Another interesting finding in this case was the presence of trisomy 8, a typical chromosomal abnormality in MDS [14], in a small subset marrow cells (9/100 by interphase FISH). The number of trisomy 8 cells decreased as myeloid/NK cell population expanded, indicating the myeloid/NK-blasts were likely to be non-trisomy 8. A similar finding was described in 2 cases of CML with transformation to Philadelphia chromosome-negative myeloid/NK-precursor leukemia [15].

In summary, although the term of “myeloid/NK cells” may be misnomer, it is diagnostically necessary to recognize such variant blasts. The first case we presented here revisited the topic of myeloid/NK cell acute leukemia, acknowledged its resemblance to APL morphologically, immunophenotypically and clinically, but also illustrated that they could be distinguished by advanced flow cytometric immunophenotyping. In the second case we first showed that myeloid/NK-cells could present as a variant of blasts in MDS. The lack of CD34, CD117 and HLA-DR may lead to underestimation of the blast number in such cases. Both cases were diagnostically challenging and required updated understanding of the surface markers in hematopoietic malignancies.

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